REMARKS

Claims 14 and 17-44 are pending and currently under consideration.

Claims 40-42, and 44 have been amended herein to correct reference to siRNA in a dependent claim in favor of proper reference to "a nucleic acid capable of mediating RNA interference." No new matter is added by virtue of the amendments. Entry and consideration of the amendments and remarks contained herein is requested.

REJECTIONS UNDER 35 USC § 112

Rejections under 35 USC § 112, first paragraph

Claims 14 and 17-44 were rejected under 35 USC §112, first paragraph, as failing to comply with the written description requirement. The Office Action states the recitation of "comprising a generation 2 to 5 dendrimer" in the claims as amended is new matter. Applicant respectfully traverses the rejection.

As discussed in the Remarks submitted in the request for continued examination filed in this application after final rejection on November 6, 2006, support for the recitation "comprising a generation 2 to 5 dendrimer" can be found in Figures 6A, 6B, and 6C, and paragraphs 0025-0027, 0075, 0102, 0103, and 0111 in the specification as filed. The structure of dendrimers is well known in the art and generally recognized as having three component parts comprising a core, an interior dendritic structure, and an exterior surface. Additionally, the term "generation" as referring to dendrimers is well understood in the art as characterizing the structure of dendrimer molecule formed following a two step synthesis strategy, where the core is regarded as generation 0, the product of a first synthesis cycle is generation 1, the product of a second synthesis cycle is generation 2, etc. See Applicant's Amendment and Response filed 11/6/06, at pages 8-10, as well as references cited and discussed therein, e.g., Stevens, M.P., *Polymer Chemistry. An Introduction, 3rd ed.*, pp. 301-303, Oxford University Press, New York, 1999; Tomalia, D.A., Birth of a New Macromolecular Architecture: Dendrimers as Quantized Building Blocks for Nanoscale Sunthetic Organic Chemistry, Alrichimica Acta, 37(2): 39-57, 2004; and Svenson, S., and Tomalia, D.A., Dendrimers in biomedical applications—reflections on the field, Adv. Drug Deliv. Rev., 57(15): 2106-2129, 2005. Thus, one skilled in the art would recognize and readily identify what generation dendrimer a structure represents by visual inspection of a depicted dendrimer structure.

Specifically, Applicant points out Figure 6A depicts a generation 5 PAMAM dendrimer, having 128 surface groups, Figure 6B depicts a generation 2 PAMAM dendrimer, having 16 surface groups, and Figure 6C depicts generation 3 PEG dendrimers having 16 or 32 surface groups, depending on the preferred core of the dendrimer (FIG. 6C-1 or FIG. 6C-2, respectively). Each of the dendrimers depicted in the Figures are readily identified by one skilled in the art, in particular with regard to what generation

dendrimeric structure each belong to, as dendrimers are generally characterized as having standard component parts comprising a core, interior dendritic structure, and an exterior surface group, and each generation expanding the size of the interior structure is easily recognized and identified. See Applicant's Amendment and Response filed 11/6/06, at pages 8-9, as well as Stevens, M.P. (1999); Tomalia, D.A., (2004); and Svenson & Tomalia (2005) cited and discussed therein. Additionally, as discussed in Applicant's prior submission, Examples 1, 2, and 7 (at paragraphs 0102, 0103, and 0111) utilize a generation 4 dendrimer in experiments to demonstrate utility and efficacy of Applicant's invention. The specific support in the Figures and Examples was included in the application as filed, as well as in the priority document provisional application as filed 11/26/2002. Applicants therefore submit the instant claims should be accorded a priority date of 11/26/2002. Thus, Applicant submits support for each of generation 2, generation 3, generation 4, and generation 5 dendrimers lies in the specification as filed and in the priority document as filed. As such, no new matter is added by virtue of the recitation of "comprising a generation 2 to 5 dendrimer." Applicant respectfully requests reconsideration and withdrawal of the rejection. Furthermore, Applicant respectfully requests the priority date of 11/26/2002 be accorded to the instant claims.

Rejections under 35 USC § 112, second paragraph

Claim 44 was rejected under 35 USC §112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. The Office Action objects to the recitation "wherein the siRNA" in the first line of the claim as lacking sufficient antecedent basis.

Claim 44 has been amended to correct inadvertent reference to "the siRNA" in favor of "the nucleic acid capable of mediating RNA interference," as recited in claim 14. Applicants have also amended Claims 40-42 to correct inadvertent reference to "siRNA" in favor of "the nucleic acid capable of mediating RNA interference," as recited in claim 14. It is believed the present amendments obviates the present rejection. Reconsideration and withdrawal of the rejection is respectfully requested.

REJECTIONS UNDER 35 USC §102

Claims 14, 20, 22-24, and 43 were rejected under 35 USC §102(e) as being anticipated by Frechet et al. (U.S. Patent No. 7,097,856). Applicant respectfully traverses the rejection.

The Office Action asserts Frechet et al. teach making dendrimers that are useful as delivery vehicles for nucleic acids, and, more specifically, teach generation 1 to 5 dendrimers as well as a specific embodiment of a generation 4 dendrimer. The Office Action states because Frechet et al. teach use of double stranded RNA, the limitation of nucleic acids capable of mediating RNAi is anticipated because

the Frechet et al. specification defines siRNA as double stranded RNA molecules. Applicant traverses the rejection and submits the disclosure of Frechet et al. does not anticipate Applicant's presently claimed invention.

Applicant respectfully points out the teaching of Frechet et al. as useful delivery vehicles, including nucleic acids, is in the context of delivery vehicles which are *conjugated* (i.e., covalently attached) to a desired agent to be delivered. For example, at column 22, line 61 through column 32, line 44 discuss general applications of use of therapeutic, diagnostic and analytical agents via conjugation to the dendrimer structure. Furthermore, at column 32, line 45 through column 36, line 43, methods for preparation of the conjugated dendrimer-agent linkages are described. Thus, Frechet et al. teaches *only* use of therapeutic, diagnostic, and/or analytic agents as a lengthy list of possibilities of agents which may be conjugated directly to the dendrimeric structures described (i.e., incorporated into the structure of the dendrimer compound) for delivery. In contrast, Applicant's presently claimed invention is directed to a *delivery mixture* (i.e., non-conjugated dendrimer-nucleic acid mixture), comprising a generation 2 to generation 5 dendrimer and a nucleic acid capable of mediating RNAi. Frechet et al. do not teach or even suggest use of the dendrimeric compounds as useful for any mixture compositions for delivery of any agent. The only teaching of use of any additional agent is as a portion incorporated into the structure of the dendrimers described and provided in Frechet et al. As such, Frechet et al. does not teach, disclose, or even suggest Applicant's present invention as claimed. Reconsideration and withdrawal of the rejection under 35 USC § 102 is requested.

REJECTIONS UNDER 35 USC §103

Rejection under 35 USC § 103(a) over Woolf, Olejnik et al., Grigoriev et al., and Yoo et al.

Claims 14, 19-20, 23-34, 38-42 and 44 were rejected under 35 USC §103(a) as being unpatentable over Woolf, Olejnik et al., Grigoriev et al., and Yoo et al. Applicant traverses the rejection.

The Office Action asserts Woolf teaches a delivery complex comprising a PAMAM complex and a double stranded RNA. Applicant respectfully disagrees and asserts the teaching of Woolf is overstated. The teaching of Woolf relating to use of a dendrimer in connection with dsRNA molecules is a single mention of PAMAM dendrimers at paragraph 203. Applicant points out this mention is in the context of a list of 14 potential cationic lipid groups which may potentially be used as complexing agents to increase cellular uptake of oligonucleotides. Additionally, paragraph 203 is but one subset of lists in twenty paragraphs of discussion of potential complexing agents Woolf suggests. See Woolf at paragraphs 201-220. Furthermore, the complexing agents are only one of several types of compounds and methods of delivery suggested in over 40 paragraphs of discussion by Woolf for delivery of oligonucleotides. See Woolf at paragraphs 186-225. Thus, in full context, the single mention of PAMAM dendrimers in

paragraph 203 is but one potential possibility in a lengthy list of possibilities suggested by Woolf for facilitating uptake of the dsRNAs described in the application. In determining selection of which of any of the agents described, one skilled in the art would have to adjust a number of variable parameters to determine what appropriate conditions, if any, would be suitable to confer a working delivery mixture. At best, the teaching of Woolf amounts merely to an invitation to experiment, or an obvious to try situation.

The Office Action states that Yoo et al. teaches a complex comprising generation 5 dendrimers and antisense oligonucleotides is efficient at delivering said oligonucleotide to cells, and variations of the ratios of dendrimer to oligonucleotide for determination of effective delivery of said complex. Applicant respectfully disagrees the teaching of Yoo et al. is sufficient to remedy the deficiency of Woolf.

The prior Office Action and Applicant's Response discussed some of the teachings and deficiencies of Yoo et al. See Office Action dated August 7, 2006 and Applicant's Amendment and Response dated November 7, 2006. The current Office Action disputes Applicant's arguments relating to the failure of Yoo et al. to demonstrate effectiveness of a generation 4 dendrimer, stating that "the 'circumstances' Yoo et al. was referring to was only the serum effects on oligonucleotide delivery by dendrimers and not the use of dendrimer 4 as a whole as a delivery agent." See Office Action dated 01/03/07 pages 14-15, bridging statement. Applicant disagrees with this characterization of the teachings of Yoo et al. The experiment that Yoo et al. references in making the conclusion demonstrates little to no activity under 0% serum conditions, as well as 30% and 50% serum conditions. Additionally, the experiment was done alongside both negative and positive controls as well as other generation dendrimers (generation 5 and generation 7 dendrimers) which were identified in this experiment and referred to throughout the article as having "moderate activity." See Yoo et al. at page 1801, column 1; and Figure 3. Yoo et al. in fact concludes " a generation 4 dendrimer was relatively ineffective under *all* circumstances [emphasis added]." See Yoo et al. at page 1801, second full paragraph. Thus, contrary to the characterization of the Office Action, the conditions described and interpreted by Yoo et al. as ineffective include non-serum, as well as serum containing conditions. Applicants therefore reiterate that Yoo et al. does not demonstrate or suggest any effective activity of a generation 4 dendrimer as a delivery agent.

Close examination of the experiments, discussion and results of Yoo et al. teaches lower generation dendrimers demonstrate only moderate activity for delivery of antisense oligonucleotide, which diminishes when lower generation dendrimer complexes are utilized. For example, as discussed above, generation 4 dendrimers do not demonstrate activity, while generation 5 dendrimers confer only moderate activity which is enhanced using a generation 7 dendrimer. Further, when compared to transfection reagents known in the art to successfully deliver oligonucleotide, generation 4, 5 or 7 dendrimers demonstrated no activity or very low activity. For example, Superfect (QIAGEN, Valencia,

CA), a proprietary but commercially available highly branched activated dendrimer useful for nucleic acid transfection, confers a high level of activity in serum free medium; and the activity conferred using LIPOFECTAMINE™ (Invitrogen Corp., Carlsbad, CA), a cationic lipid based transfection agent used as a standard in the art, is even more improved over the effects demonstrated by Superfect. For instance, generation 5 dendrimer confers an average activity of less than about 25,000 RLU/μg Protein, as compared to about 150,000 RLU/μg Protein for LIPOFECTAMINE™. This activity increases to just over about 30,000 RLU/μg Protein using a generation 7 dendrimer and up to about 100,000 RLU/μg Protein when Superfect is utilized. See Yoo et al., Figure 3 and page 1801, left column, second full paragraph. Surprisingly, Yoo et al., characterizes the levels of generation 5 and generation 7 as "moderate," however, when characterizing the levels of activity in the presence of serum, an average activity for LIPOFECTAMINE™ decreasing to about 5,000 RLU/μg Protein is determined to be negligible activity, while levels of 12,500 to 7,000 RLU/μg Protein are characterized as "maintaining moderate activity" to "some activity." For example, Yoo et al states, "generation 5 and 7 dendrimers showed moderate activity (compared to LIPOFECTAMINE™ or Superfect) in serum free medium, but maintained a higher relative degree of effectiveness in the presence of high concentrations of serum." Compare Yoo et al., Figure 2, and at page 1801, first column. Applicant points out the levels recognized by Yoo et al. as "moderate activity" may in fact be interpretable as little to no activity. Yoo et al. states "Although Superfect showed a substantial loss in relative potency, the actual levels of luciferase attained by treatments using generation 5 or 7 dendrimers or Superfect were not statistically different." See Yoo et al., at page 1801, left column. However, the activity levels recognized as a substantial loss of activity (nearly 95% loss in activity), is interpreted as maintaining "moderate activity" by Yoo et al. Applicant submits such interpretation is debatable as to the effectiveness of the teaching conferred by the experiments exemplified in Yoo et al. The stated goal of the study of Yoo et al. was to identify effective delivery agents as alternatives to known agents which could confer effective delivery over the limitations of known agents such as cationic lipid complexes (e.g., LIPOFECTAMINE™). For example, Yoo et al. states: "Unfortunately, most of these agents, including many commercially available cytofectins, are rather poor in delivering oligonucleotides to cells in the presence of serum, as compared to effects attained under serum-free cell culture conditions. Thus, these agents are not likely to be effective *in vivo*, where plasma proteins are abundant." See Yoo et al., at page 1799, first paragraph of Introduction. Similarly, the comparably very low levels of activity obtained by Yoo et al. using lower level dendrimer complexes (e.g., generation 7 and particularly generation 5) may be interpreted by one skilled in the art as having limited utility for delivery agents, thus rendering the compositions and uses described and claimed in the present invention non-obvious.

In addition to increasing dendrimer generation correlating with improved delivery effect, Yoo et al. teach improved activity correlates with increased oligonucleotide concentration and increased charge ratio of prepared dendrimer complexes. See Yoo et al., at Figures 2-5 and 7, and page 1801, left column, first full paragraph, through second column, first paragraph, and at page 1803, entire Discussion. Thus, in assessing activity of such complexes, numerous factors including dendrimer generation, oligonucleotide concentration, charge ratio, and presence of serum are factors which require optimization. The combination of numerous changes in each of these factors amounts to a large number of possibilities capable of being explored. Such a large amount of possibilities is beyond routine experimentation and optimization, rather amounts to attempts to identify working combinations and mere experimentation.

When viewed alongside the diminished activities of LIPOFECTAMINE™ or Superfect in the presence of serum, which Yoo et al. acknowledges as non-preferable delivery agents due to such limitations, one would not necessarily identify such low levels of dendrimer complex activity as being obviously useful for delivery of an alternative oligonucleotide, particularly when additional modification and optimization is required. Because the effectiveness of generation 5 or 7 dendrimers is comparably very low as compared to those agents known and recognized to confer efficient delivery and activity (e.g., Superfect or LIPOFECTAMINE™), one would not necessarily expect any change in nucleic acid would yield results which are interpretable as demonstrating only very minimal activity, much less any efficacy. Furthermore, because (1) activity is dependent on the dendrimer generation; (2) decreased activity correlates with lower generation dendrimer; (3) generation 5 dendrimers confer only minimal activity in optimized circumstances, (4) generation 4 dendrimers demonstrated no effective activity; and (5) additional requirements for optimization such as oligonucleotide concentration and increased charge ratio affect activity of dendrimer complexes, one skilled in the art would not expect to identify a generation 5 dendrimer in combination with a replacement oligonucleotide useful for any level of efficient activity.

Applicant further submits the Office Action overstates the teaching of Yoo et al. in making assertions that one skilled in the art would have been motivated to make a delivery mixture comprising siRNA oligonucleotides and dendrimers, and would undertake only routine optimization of ratios, and concentrations to achieve the expected success. For example, Yoo et al. is characterized as teaching "a complex comprising a generation 5 dendrimer and an antisense wherein the complex *displayed substantial activity* for the delivery of said oligonucleotide", and further, "Yoo et al. teach various ratios of dendrimer to nucleic acid for optimization." See Office Action dated 01/03/07 at page 10, second paragraph (emphasis added). Yoo et al. states: "generation 5 dendrimer displayed substantial activity for the delivery of oligonucleotides in serum-free medium, had moderate activity under standard cell culture conditions (10% serum), and maintained some activity even in the presence of 70% serum." See Yoo et al., at page 1803, second paragraph. However, Applicant submits this discussion of Yoo et al. refers

solely to interpretation of the relative activity result of generation 5 in the absence and presence of serum, without comparison to known industry standards, and not the overall conclusion of Yoo et al. with regard to the activity assessment of generation 5 dendrimers. As discussed above, the interpretation and conclusions of the authors Yoo et al. was "that PAMAM dendrimers form stable complexes with oligonucleotides, and are *moderately* efficient in delivering oligonucleotide even in the presence of high concentration of serum protein." See Yoo et al., at page 1799, last paragraph of Introduction. Furthermore, Yoo et al. suggest their results are only a beginning for further experimentation. For example, in the Discussion, Yoo et al states the results of the experiments described "makes these agents attractive candidates for futher development in the context of *in vivo* application of antisense oligonucleotides." See Yoo et al., at page 1803, last sentence of Discussion.

In summary, Applicant submits the teaching of Yoo et al. instructs one skilled in the art that generation 5 or generation 7 dendrimers may have potential applications for delivery of antisense oligonucleotide activity to cells, but that effective delivery and activity is dependent upon concentration of oligonucleotide, charge ratio of oligonucleotide to dendrimer, and generation of dendrimer used. Yoo et al. acknowledges each of these conditions, for example, at page 1803, second full paragraph, stating the "concentration of oligonucleotide, the charge ratio of nucleotide to dendrimer, and the generation of the dendrimer all influenced the antisense delivery effect." Finally, the presence of increasing amounts of serum negatively influences the activity of the complexes even in the presence of different generation dendrimer complex, or optimized concentration, and charge ratio. See e.g., Yoo et al., Figures 1-7. Because of the limited demonstration of highly efficient activity and efficacy, combined with the numerous parameters necessary for modification and optimization, the teaching of Yoo et al. amounts to no more than a suggestion to one skilled in the art for further experimentation in the field. Even assuming the combination teachings of Woolf and Yoo et al. would be obvious to one skilled in the art, the combined teaching provides nothing more than an obvious to try situation.

Neither Olejnik et al. nor Grigoriev et al. provide any teaching or suggestion regarding a delivery mixture comprising a generation 2 to 5 dendrimer and a nucleic acid capable of mediating RNA interference. As acknowledged in Applicant's prior response, Olejnik discloses the design, synthesis, and evaluation of a non-nucleosidic photocleavable biotin phosphoramidite (PCB-phosphoramidite) for simple purification and phosphorylation of oligonucleotides; and Grigoriev discloses use of psoralen-oligonucleotide conjugates useful for triple helix formation and cross-linking to DNA following UV irradiation. None of Olejnik et al. or Grigoriev et al., whether alone or in any combination with Woolf and Yoo et al., provide any teaching to suggest to one skilled in the art to produce a delivery mixture comprising a generation 2 to 5 dendrimer and a nucleic acid capable of mediating RNA interference. Furthermore, one skilled in the art would have no reasonable expectation that such a composition would

provide success, much less result in any improved results as compared to those demonstrated using generation 5 dendrimers and antisense oligonucleotides in Yoo et al. Thus, neither Olejnik et al. or Grigoriev et al. remedy the deficiencies of Woolf and Yoo et al. Reconsideration and withdrawal of the rejection under 35 USC §103(a) is thus respectfully requested.

Secondary Considerations

Whether an art is predictable or whether the proposed modification or combination of the prior art has a reasonable expectation of success is determined at the time the invention was made. See MPEP, 2143.02, citing *Ex parte Erlich*, 3 USPQ2d 1011 (Bd. Pat. App. & Inter. 1986). Furthermore, failure of others is a secondary consideration or indicia of nonobviousness. *Graham v. John Deere Co.*, 383 U.S. 1, 17-18. As discussed in Applicant's prior response, attempts to substitute siRNA for antisense oligonucleotides as suggested by the Office Actions have demonstrated that success was certainly not expected or obvious. In support of Applicant's argument, Kang et al. was submitted and discussed. See Amendment and Response dated 11/07/06, and Kang, H., et al., *Pharm Res*. 22:2099-2106 (2005). The Office Action asserts the opposite conclusion: the teaching of Kang is supportive that the invention as a whole would be *prima facie* obvious to one skilled in the art at the time the invention was made. Applicant respectfully disagrees.

Applicant agrees with the Office Action that Kang demonstrates formation of strong dendrimer-oligonucleotide complex formation, and that Kang demonstrates similar cellular distribution of siRNA and antisense which is delivered to cells. However, formation of complexes and/or minimal delivery and distribution in cells alone along does not equate to sufficient delivery of oligonucleotide for successful activity within a cell. Kang acknowledges this, for example, in the Conclusion discussion of the differential effects found with antisense and siRNA:

> *Another observation is that PAMAM dendrimers or their conjugates seem more effective in delivering antisense oligonucleotides than siRNA. Thus, when compared to LIPOFECTAMINE™ 2000 as a positive control, the dendrimers were comparably effective in reducing P-glycoprotein expression when conveying a phosphorothioate antisense oligonucleotide. However, when a siRNA was used the cationic lipid was much more effective than the dendrimers in promoting reduction of P-glycoprotein expression. The basis for this is not obvious; both types of oligonucleotides form strong complexes with both types of carriers. In addition, the fluorescence microscopy images did not reveal any major differences in the subcellular distribution of antisense or siRNA delivered via dendrimers; in both cases the oligonucleotides were primarily associated with vesicles with some diffuse distribution in the cytosol and nucleus. However,*

> *apparently subtle differences in the delivery process differentially affect the distinct mechanisms of message degradation employed by siRNA vs. antisense. In summary, this study confirms previous results that PAMAM dendrimers are moderately effective agents for delivery of antisense oligonucleotides. However they are relatively less effective for delivery of siRNA.*

See Kang at page 2105, last paragraph through page 2106 first paragraph. Thus, while the Office Action characterizes the results of Kang as evidence that one would expect an siRNA molecule to behave identical to an antisense molecule in dendrimer-nucleotide complexes since the molecules "are both nucleic acids that encounter the same delivery problems" (see Office Action dated 01/03/07 at page 15), the conclusions and reported results of Kang are directly contrary to this interpretation. In fact, Kang's interpretation of the data and conclusion is that "*gene expression was partially inhibited by the antisense-BPT complex and weakly inhibited by the siRNA-BPT complex when both were tested at nontoxic levels of dendrimer...Dendrimer-oligonucleotide complexes were moderately effective for delivery of antisense and only poorly effective for delivery of siRNA*". See Kang at page 2099, Abstract at Results.

Applicant submits the showing of failure of success by Kang et al. is more than mere failure of another of skill in the art to carry out combined teachings in the prior art. The experiments carried out in Yoo et al. and Kang et al. were in fact done in the same laboratory of Dr. Rudolph Juliano at the University of North Carolina, Department of Pharmacology, and Dr. Juliano is a last author listed on both publications. See Yoo et al. and Kang et al. Hoon Yoo was a previous post-doctoral fellow working in Dr. Juliano's lab, and Hyunmin Kang is a present post-doctoral fellow working in Dr. Juliano's lab. See Rudolph L. Juliano laboratory description web page at http://www.med.unc.edu/pharm/faculty/juliano.htm; and Juliano lab members web page at http://www.med.unc.edu/pharm/rjlabpg/previous.htm, copies of which are enclosed. Thus, even when no speculation about combining references of another, or interpretation as to what may be obvious to one of ordinary skill in the art in view of the teaching of Yoo et al. is required; when the same lab substituted siRNA for antisense in the experiments of Yoo et al., results obtained when siRNA was used were worse, not equal to, and definitely not better than results demonstrated by Yoo et al.

Thus, because of the differential activities demonstrated, as well as the poor effects demonstrated using siRNA, Kang evidences that a substitution of an siRNA oligonucleotide for an antisense oligonucleotide in dendrimeric complexes in a delivery mixture would not be obvious, or be expected to achieve success. Applicant submits that, when the evidence is considered as a whole, the combination of Woolf and Yoo et al. would not be obvious to one skilled in the art, but at most provide an invitation to experiment. Furthermore, in view of the secondary consideration of failure of others and limited

effectiveness demonstrated by Yoo et al., one skilled in the art would not have a reasonable expectation of success that such a combination would be effective.

Applicant submits additional secondary considerations of non-obvious surprising results have been demonstrated in the disclosure of the present application that render the invention as claimed non-obvious. Evidence of unexpected results can be used to rebut a *prima facie* case of obviousness. See, e.g., *In re Peterson*, 315 F.3d 1325, 1330 (Fed. Cir. 2003). Applicant submits the delivery mixture comprising a generation 2 to generation 5 dendrimer and a nucleic acid capable of mediating RNA interference as described and currently claimed confers delivery and efficacy which are far superior to those compositions described in Yoo et al. For example, compositions prepared and used according to the present application demonstrated delivery of nucleic acid at levels comparable to or even higher that levels achieved using a LIPOFECTAMINE™ standard. Additionally, the siRNA activity correlated directly with the efficiency of delivery of nucleic acid. See Examples 1 and 2, paragraphs 102-103, and Figures 1 and 2 of the application as published. These levels demonstrate activity which is far improved as compared to the experiments described in Yoo et al., and would not be expected in view of the teaching of Yoo et al., whether alone or in combination with one or more of the additionally cited references.

As discussed in Applicant's prior response, the activity of the complexes does not appear to correlate consistently with increased concentration of dendrimer. Rather, dendrimer concentrations below 20μg/mL or above 40μg/mL were found to be less effective in conferring efficient cell uptake and RNAi activity. See Examples 1 and 2, paragraphs 102-103, and Figures 1 and 2 of the application as published. Furthermore, Applicant has found the cellular localization conferred by the delivery complexes is critical for efficient activity of the delivery complexes and RNAi activity in the cell. For example, delivery complexes comprising an effective amount of dendrimer localize siRNA to perinuclear regions of the cytoplasm as well as nuclear regions. Higher amounts of dendrimer complexes, which were less efficient for delivery, appeared to disrupt cellular localization, indicating that subcellular localization of siRNA is important for RNAi activity. See Examples 6 and 7, paragraphs 110-111, and Figures 8 and 9 of the application as published.

Finally, effective delivery of the dendrimer and nucleic acid mixtures provided and presently claimed do not confer toxic effects to cells as reported by Yoo et al. In sum, the efficacy of the delivery mixture comprising a generation 2 to 5 dendrimer and a nucleic acid capable of mediating RNA interference disclosed and claimed in the present invention provides far superior properties to the delivery mixture described in Yoo et al. such that the presently claimed invention would not be obvious and taught by any of Woolf, Yoo et al., Olejnik et al., and Grigoriev et al., whether alone or in combination. Thus,

even if a *prima facie* case of obviousness had been made, the unexpected superior results demonstrated by Applicant are secondary considerations sufficient to rebut such a showing.

In view of the above, Applicant submits the invention as provided and presently claimed would not be obvious to one skilled in the art; as such the rejection under 35 USC 103(a) should be withdrawn. Reconsideration and withdrawal of the rejection is respectfully requested.

Rejection under 35 USC § 103(a) over Yoo et al., in view of Hammond et al., Tuschl et al., and McManus et al..

Claims 14, 17-24 and 32-44 were rejected under 35 USC §103(a) as being unpatentable over Yoo et al. in view of Hammond et al., Tuschl et al., and McManus et al.

The Office Action states that Yoo et al. teach a delivery mixture comprising a PAMAM dendrimer and an antisense nucleic acid capable of inhibiting gene expression. See Office Action at page 10, second paragraph. The Office Action further states a complex comprising a generation 5 dendrimer described in Yoo et al. displayed "substantial activity" for the delivery of the oligonucleotide. The Office Action admits that Yoo et al. do not teach a dsRNA or an RNA precursor capable of mediating RNAi.

For the reasons discussed above, when considered as a whole, one skilled in the art would not arrive at the conclusion that Yoo et al. provides sufficient teaching such that one would expect that replacing the antisense molecule with a dsRNA (or any other nucleic acid capable of mediating RNA interference) would be effective for mediating RNA interference. Given the knowledge in the field at the time of the invention, one skilled in the art would not consider such a combination to be obvious, as it was not predictable whether such a replacement would be successful.

Alone or in combination, Hammond et al., Tuschl et al., or McManus et al. do not remedy the deficiency of Yoo et al. As discussed by the Examiner, Hammond et al., Tuschl et al., and McManus et al. teach use of dsRNA, siRNA, and shRNA and microRNA, respectively, as alternative approaches to gene silencing and inhibition of gene expression. The Office Action maintains that one skilled in the art would readily substitute a dsRNA, siRNA, shRNA or microRNA for an antisense molecule in a delivery mixture of Yoo et al. in view of each of Hammond et al., Tuschl et al., and McManus et al. The reason the Office Action proffers is that substitution would be preferred because Hammond et al. teach that RNA interference is more efficient than antisense for probing gene function and for inhibiting gene expression, and since the molecules utilized are nucleic acids, one would expect to encounter similar issues in delivery to cells as with antisense oligonucleotides. Thus, the Examiner concludes that one would be motivated to use a delivery mixture comprising a dendrimer because the goal for siRNA is optimal delivery of the siRNA and enhanced cellular uptake by the cells.

The Office Action cites Hammond et al. at page 110, column 1, as teaching that although "antisense methods ... are relatively straightforward techniques for probing gene function; however these methods have consistently suffered from questionable specificity and incomplete efficacy." See Office Action dated 01/03/07 at page 10, bottom paragraph, and Hammond et al. at page 110, column 1. Applicant points out that this reliance on the stated deficiency of antisense as a basis for routine substitution of molecules suggested by Hammond et al., Tuschl et al., and McManus et al. is not well grounded, and does not consider all of the underlying reasons for the deficiencies of antisense. The reasons for the deficiencies of antisense in probing gene function and inhibiting gene function support a lack of reasonable expectation of success for substitution of any other nucleic acid described in Hammond et al., Tuschl et al., and McManus et al.

The deficiencies of antisense, such as questionable specificity and incomplete efficacy, are recognized by one skilled in the art to include not only to the efficiency of antisense nucleic acids *per se*, but also to efficient target delivery of nucleic acids. For example, a 2002 review of antisense discusses the basic concepts and mechanisms of antisense, as well as reasons for the shortcomings of the technology. See Dias, N., and Stein, C.A., *Mol. Cancer Ther.* 1: 347-355 (2002), a copy of which is enclosed herewith. Because Dias and Stein is a review article published in 2002, the publication and discussion of Dias and Stein evidences the knowledge and skill in the art at or around the time of the making of the invention; and is particularly relevant to the determination of obviousness of the invention, and whether the combination of Yoo et al., Hammone et al., Tuschl et al., and McManus et al. would have a reasonable expectation of success.

At pages 350-351 Dias and Stein discuss issues associated with delivery of antisense, including delivery mixtures comprising PAMAM dendrimers and other cationic delivery systems. Cationic delivery systems use the endosomal pathway to delivery oligonucleotides into the cell. Often "helper" molecules are necessary to facilitate escape of the oligonucleotides from the endosome to allow transport to the nucleus. Additionally, due to the charged nature of the cationic polymers, they "appear to cause endosomal rupture via a "molecular sponge" mechanism, tend to be somehat toxic and are less commonly used that are cationic liposomes." See Dias and Stein at page 351, second and third paragraphs. Dias and Stein follow this section with rationale for exploration of alternative improved delivery approaches, stating: "All of these cationic delivery systems internalize oligonucleotides via an endocystotic mechanism. To avoid the resulting compartmentalization problems, consideration has been given to modulating plasma membrane permeability." In conclusion, Dias and Stein summarize: "the optimal use of antisense oligonucleotides in the treatment of disease requires the resolution of problems relating to effective design, enhanced biological activity, and efficient target delivery." See Dias and Stein at page

352, last paragraph. Thus, the shortcomings of antisense relate not only to the nucleic acid molecules themselves, but also to effective and efficient delivery in the cell so the nucleic acid can function.

Applicant submits the reasons the Office Action lists as supportive motivation to substitute a nucleic acid capable of mediating RNA interference for an antisense oligonucleotide in the delivery mixture described in Yoo et al. are in fact supportive of a lack of expectation of success that such a substitution would necessarily work. Since the problems reported for cationic polymers (e.g., PAMAM dendrimers, etc.) such as toxicity and localization would be expected to be conferred on any nucleic acid utilized in such mixtures, it would not be obvious that a substitution of one type of nucleic acid for another would be successful in such application.

When considered as a whole, Applicant submits the teachings of Yoo et al. in view of Hammond et al., Tuschl et al., and McManus et al., whether alone or in combination, can only suggest that one skilled in the art try to substitute any of a dsRNA, siRNA, shRNA or microRNA for antisense in a delivery mixture of Yoo et al. Hammond et al., Tuschl et al., or McManus et al., alone or in combination with Yoo et al. do not remedy the deficiencies of Yoo et al. alone. A mere suggestion to experiment or obvious to try is not the standard supportive of an obviousness rejection under 35 USC § 103.

Secondary considerations

As discussed above in the previous rejection under 35 USC § 103, secondary considerations have been demonstrated which support rebuttal of obviousness if a *prima facie* case were presented.

First, as discussed above, Yoo et al. failed to demonstrate any activity of a delivery complex comprising a generation 4 dendrimer and an antisense oligonucleotide. Furthermore, as also discussed above, when the replacement proposed by the Office Action (i.e., replacement of an antisense oligonucleotide with an siRNA) was carried out in the same laboratory as the experiments and results described in Yoo et al., the results using siRNA were not as good as the results demonstrated using antisense. See Kang et al. Failure of others is one of the secondary considerations or indicia of nonobviousness. *Graham v. John Deere Co.*, 383 U.S. 1, 17-18 (1966). Thus, the failed results of Yoo et al. utilizing a delivery complex comprising a generation 4 dendrimer, as well as Kang et al. utilizing a delivery complex comprising a dendrimer and an siRNA support a conclusion of non-obviousness of a delivery mixture comprising a generation 2 to 5 dendrimer and a nucleic acid capable of mediating RNA interference.

Second, Applicant's showing of evidence of unexpected results can be used for rebuttal if a *prima facie* case of obviousness were presented. See secondary considerations and evidence of unexpected superior results discussed *supra*. Applicant submits for the same reasons as discussed above, the delivery

mixture comprising a generation 2 to generation 5 dendrimer and a nucleic acid capable of mediating RNA interference as described and currently claimed confers delivery and efficacy which are far superior to those compositions described in Yoo et al., and would therefore be considered non-obvious.

In view of the above, Applicant submits the present rejection is not proper. Applicant requests reconsideration and withdrawal of the rejection under 35 USC 103(a).

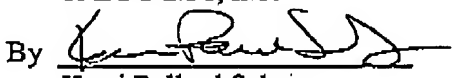
Entry and consideration of the amendments and remarks contained herein is respectfully requested. If at any time a telephone discussion would assist the Examiner and/or expedite prosecution, the Examiner is invited to contact the undersigned.

This paper is being filed timely as it is being filed with a petition for a one month extension of time, and the associated fees. It is believed no additional fees and/or extensions of time are required. In the event any additional extensions of time, fees and/or credits are necessary, the undersigned hereby authorizes the requisite fees to be charged and/or credited accordingly to Deposit Account No. 50-1582.

Respectfully submitted,

May 3, 2007

MIRICK, O'CONNELL, DEMALLIE
& LOUGEE, LLP

By 

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